REMARKS

The above amendments and these remarks are responsive to the Office action dated November 3, 2003 and support the accompanying Request for Continued Examination as a submission under 37 C.F.R. § 1.114(c). Claims 94-105 and 108-115 are pending in the application. In the Office action, the Examiner rejected claims 94-105 and 108-115 as being indefinite under 35 U.S.C. § 112 and/or obvious under 35 U.S.C. § 103(a). Applicants traverse these rejections. Applicants contend that the rejected claims are neither indefinite nor obvious. Nevertheless, to reduce the number of issues under consideration, and to expedite the issuance of a patent, applicants have amended claims 94 and 115 to remove the phrase "operation of the enzyme." Moreover, to provide further support for their arguments that claims 94-101, 108, 110, and 112-115 are nonobvious, applicants have presented additional experimental results showing that the subject matter of these claims provides significant unexpected benefits. These benefits are not suggested by the prior art and provide significant, practical advantages over previous assays. Accordingly, in view of their Request for Continued Examination, applicants respectfully request reconsideration of the rejected claims, and prompt issuance of a notice of allowance.

I. Request for Continued Examination

Applicants are submitting herewith a Request for Continued Examination (RCE) under 37 C.F.R. § 1.114. This Request complies with the requirements of 37 C.F.R. § 1.114. In particular:

(i) Prosecution in the application is closed, since the last Office action was a final Office action under 37 C.F.R. § 1.113.

(ii) The Request is accompanied by a submission as set forth at 37 C.F.R. § 1.114(c), specifically, the amendments, remarks, and arguments set forth herein.

(iii) The Request is accompanied by the fee set forth at 37 C.F.R. § 1.17(e).

Accordingly, applicants respectfully request grant of their Request for Continued Examination.

II. Supplemental Information Disclosure Statement

Applicants will be filing a Fifth Supplemental Information Disclosure Statement (IDS) in the next few days to disclose additional references. Applicants respectfully ask the Examiner to consider the IDS, and the references cited therein, in reviewing this communication.

III. Claim Rejections – 35 U.S.C. § 112

The Examiner rejected claims 94-105 and 108-115 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter that applicants regard as the invention. Applicants traverse these rejections. Applicants believe that the pending claims are definite. Nevertheless, to reduce the number of issues under consideration, and to expedite the issuance of a patent, applicants have amended claims 94 and 115 to remove the phrase "operation of the enzyme."

A. Claim 94

The Examiner rejected claim 94 under 35 U.S.C. § 112, second paragraph, contending that the phrase "product of the operation of the enzyme" is vague and indefinite. In particular, the Examiner stated that the "kind of 'operation'" (e.g., phosphorylation or decyclization) to which applicants referred was unclear. Applicants

disagree. The preamble of claim 94 includes the phrase "an enzyme that operates on a substrate to form a product." Accordingly, the enzyme may perform any operation that forms a product from a substrate. Applicants suggest that one of ordinary skill in the art would clearly understand the meaning and breadth of the term "operation" in this context of product formation from substrate, based on an understanding of the term "enzyme." In particular, one of ordinary skill in the art would understand "operation" to include, but not be limited to, the examples of phosphorylation and decyclization suggested by the Examiner, as well as dephosphorylation, cyclization, reduction, oxidation, addition, subtraction, ligation, cleavage, isomerization, folding, and/or unfolding, among others, of a substrate to form a product. Applicants thus assert that the phrase "product of the operation of the enzyme" is not indefinite. Nevertheless, to more particularly point and distinctly claim aspects of their invention, applicants have amended claim 94 to delete the phrase "operation of the enzyme," as follows:

> 94. (Currently amended) A method of detecting the activity of an enzyme that operates on a substrate to form a product in a sample, comprising:
>
> contacting the substrate with the enzyme in the sample;
>
> contacting the sample with a binding partner that specifically binds to the substrate or to [[a]] **the** product ~~of the operation of the enzyme~~, but not to both, wherein the binding partner includes gallium that is required for binding between the binding partner and the substrate or the product;
>
> detecting a response, based on luminescence polarization, indicative of the extent of binding between the substrate or the product and the binding partner without separating the bound substrate or product from the unbound substrate or product; and
>
> correlating the response with the activity of the enzyme.

The phrase "**the** product", which replaces "a product of the operation of the enzyme" in the second contacting step of the claim, as amended, clearly and definitely refers back to the "product" that appears in the preamble of the claim. The types of enzymatic

reactions covered by this claim, and the associated enzymes, substrates, and products, are those that may be assayed using a binding partner that includes gallium that is required for binding between the binding partner and the substrate or the product. These enzymatic reactions include, but are not limited to, those listed above.

B. Claim 115

The Examiner also rejected claim 115 under 35 U.S.C. § 112, second paragraph, contending as with claim 94 that the phrase "product of the operation of the enzyme" is vague and indefinite. Applicants again disagree. Nevertheless, to more particularly point and distinctly claim aspects of their invention, applicants have amended claim 115 to remove the term "operation of the enzyme." In particular, amended claim 115 now recites, in pertinent part, "the step of contacting the sample with a binding partner" without reference to "operation of the enzyme," as follows:

> 115. (Currently amended) The method of claim 94, wherein the step of contacting the substrate with the enzyme precedes the step of contacting the **sample** ~~substrate or any product produced by operation of the enzyme~~ with a binding partner.

C. Conclusion

Applicants believe that claims 94 and 115, as amended, are definite. Thus, for at least this reason, applicants respectfully request withdrawal of the rejections under 35 U.S.C. § 112, second paragraph.

IV. Claim Rejections – 35 U.S.C. § 103

The Examiner rejected claims 94-101, 108, 110, and 112-115 under 35 U.S.C. § 103(a) as being unpatentable over Nikiforov (U.S. Patent No. 6,472,141) in view of Posewits et al. (Anal. Chem. 7:2883-92 (1999)). Applicants traverse these rejections.

The Examiner stated that it would have been obvious to one having ordinary skill in the art at the time the invention was made to replace the conventional metal ions for capturing phosphopeptides as taught by Nikiforov with the Ga ion as taught by Posewitz et al. with a reasonable expectation of success.[1] Specifically, the Examiner asserted that Posewitz provides motivation for replacing iron, as disclosed by Nikiforov, with gallium. Applicants disagree. In particular, applicants have found at least three significant unexpected benefits[2] to using gallium in luminescence polarization assays that are neither taught nor suggested by Nikiforov, Posewitz, or any other prior art reference of record. First, gallium, in contrast to iron, enhances intensity, instead of quenching intensity, after associating with luminescent assay components. Consequently, assays employing gallium can be performed much more quickly and with much less statistical noise than assays employing iron. Second, assays employing gallium, in contrast to assays employing iron, have a much greater dynamic range of polarization. Consequently, assays employing gallium are much more robust and easy to perform than assays employing iron, if the latter can be performed at all. Third, assays employing gallium to bind product can better distinguish the existence of product in a mixture of substrate and product, relative to an iron reagent, since product bound to the gallium reagent will contribute more rather than less to the total polarization. The first advantage, at least, was presented in the application, as filed.[3] Moreover, all three advantages augment the teach-away argument that applicants maintain from their previous response to Office action.[4] Experimental results detailing these unexpected

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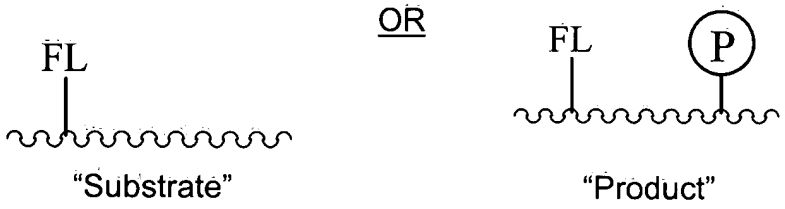
[1] Office Action (November 3, 2003), page 4, first full paragraph.
[2] See MPEP §716.02.
[3] See, e.g., Application, page 33, lines 8 and 9.
[4] See Response to Office Action (July 30, 2003), page 9.

benefits are described in a Declaration from Dr. Richard Sportsman, an expert on luminescence polarization assays, and one of the inventors on the subject application. This declaration is included as Exhibit A, and the results are summarized below.

A. Overview of Experimental Procedure

Applicants performed a series of binding assays to compare reagents that include iron ("iron reagent") or gallium ("gallium reagent"). Each binding assay included either a fluorescent substrate (a luminophore-labeled, nonphosphorylated peptide; "S") or a fluorescent product (a phosphorylated form of the peptide; "P"), as follows:



The product corresponds to a product that would be produced by operation of a suitable kinase enzyme on the substrate. The fluorescent substrate or product was incubated with the iron reagent or the gallium reagent. Then, the effect of each metal on luminescence intensity and polarization from the substrate or product was measured. Accordingly, these binding assays with gallium correspond to an embodiment of the invention for detecting kinase enzyme activity in which there is no enzyme activity (substrate (S) only) or substantial enzyme activity (product (P) only). Alternatively, by reversing the substrate and product designations of these peptides, the results correspond to an embodiment of the invention for detecting phosphatase enzyme activity in which there is substantial enzyme activity or no enzyme activity, with similar

conclusions. Results corresponding to embodiments of the invention in which there are intermediate enzyme activities also are described below.

B. Advantage 1: Unexpected Benefits on Intensity Measurements

The experimental results presented in the declaration show that gallium provides at least one significant unexpected benefit over iron for measurements of luminescence intensity. Importantly, measurements of luminescence intensity underlie all measurements of luminescence polarization.[5]

Figure 1 of the declaration, which is reproduced below, shows results of total luminescence intensity measurements in relative fluorescence units (RFU), as a function of added metal (iron reagent or gallium reagent) and peptide phosphorylation state (P or S) in the binding assays:

[5] The relationship between polarization and intensity is expressed by the following equation:

$$P = \frac{I_{||} - I_{\perp}}{I_{||} + I_{\perp}}$$

Here, P is the polarization, $I_{||}$ is the intensity of luminescence polarized parallel to the polarization of the excitation light, and $I_{\perp}$ is the intensity of luminescence polarized perpendicular to the polarization of the excitation light, all following excitation with polarized light. Thus, measurements of polarization are only as good as the underlying measurements of intensity. See, e.g., Application, page 52, lines 3-8.

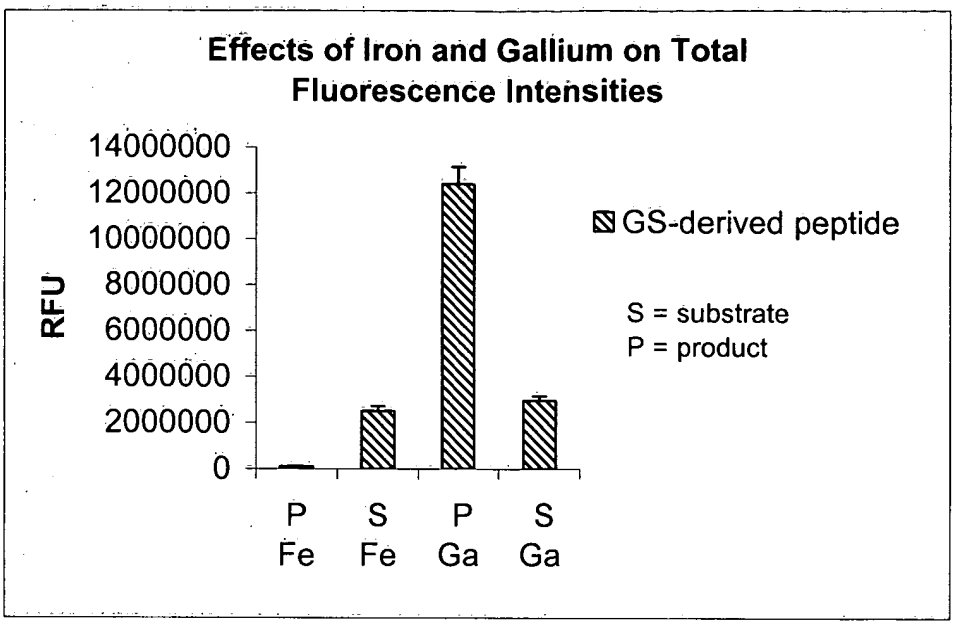


Figure 1

The luminescence intensity (or brightness) generally was comparable in assays of the substrate (S) performed with either the iron reagent or the gallium reagent. These results are consistent with little or no binding of each reagent to this substrate, and thus little or no effect on the intensity of luminophore associated with this substrate. In contrast, the luminescence intensity (or brightness) differed dramatically according to which of these reagents was present in assays of the product (P). In particular, the intensity was about one-hundred-fold higher in assays of the product performed with the gallium reagent relative to assays of the product performed with the iron reagent. This dramatic difference in intensity was produced, in part, by an approximately four-fold increase in the intensity of the product relative to substrate (S) for the gallium reagent, and, in part, by a more than twenty-fold decrease in the intensity of the product relative to substrate for the iron reagent. Therefore, in contrast to the iron reagent, which quenched luminescence intensity substantially when present with product and its

associated luminophore, the gallium reagent not only did not quench but actually enhanced this intensity.

These dramatic differences in intensity between the iron reagent and the gallium reagent translate into dramatic differences in the timing and sensitivity of assays performed with these reagents. Polarization assays on large numbers of samples are configured to be analyzed as fast as possible, to maximize throughput. For example, the SmartRead™ system employed in polarization readers produced by Molecular Devices Corporation is designed to collect data on each sample until a threshold number of photons (or a timeout period) has been reached, and then move on to the next sample. This threshold number of photons typically is selected to correspond to a minimally acceptable signal-to-noise ratio for data analysis.[6] Thus, due to the opposing effects of iron and gallium on brightness, it would take about 100 times as long to collect comparable light per sample with the iron reagent as with the gallium reagent. This difference literally is the difference between practical and impractical in high-throughput drug screening, since assay measurements that take 20-40 milliseconds to perform with gallium would take a completely unacceptable 2-4 seconds to perform with iron! Moreover, in any context, this difference means that for a given measurement time the signal strength (and thus the signal-to-noise ratio) will be significantly higher in gallium-based polarization assays than in iron-based polarization assays.

The dramatic intensity advantages of gallium relative to iron are not obvious. To the contrary, metals are well-known luminescence quenchers (i.e., extinguishers). Indeed, Pierce Biotechnology recently began selling a kinase assay system in which

[6] The signal-to-noise ratio of the intensity in photon processes is proportional to the square root of the number of photons collected.

enzyme activity is observed using luminescence quenching that accompanies interaction of a fluorescently labeled phosphorylated peptide with iron; see Exhibit B.[7] Specifically, in the Pierce assay, luminescence intensity decreases monotonically with increasing phosphorylation, increasing kinase concentration, and increasing time, all reflecting increasing association of iron with the luminophore.[8] In contrast, applicants have discovered that gallium, unlike iron, not only does not quench but instead actually enhances intensity when bound to a luminophore. None of the references of record, including Nikiforov and Posewitz, teach or suggest this unexpected and patentable benefit of using a binding partner including gallium in polarization assays.

C. *Advantages 2 and 3: Unexpected Benefits on Polarization Measurements*

The experimental results presented in the declaration also show that gallium provides at least two significant unexpected benefits over iron in distinguishing product from substrate in polarization assays.

First, gallium-based polarization assays have significantly higher dynamic ranges than iron-based polarization assays, enhancing the speed at which assays can be performed. (This enhancement in speed comes in addition to the enhancement described above relating to differences in intensity.) Figure 2 of the declaration, which is reproduced below, shows a plot of luminescence polarization (mP) measured as a function of added metal (iron or gallium) and peptide phosphorylation state:

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[7] See, e.g., Pierce IQ® Assay Platform: Technical Handbook (Pierce Pub. No. 1600963) (August 2003). This handbook is included with this Response as Exhibit B.

[8] Id.

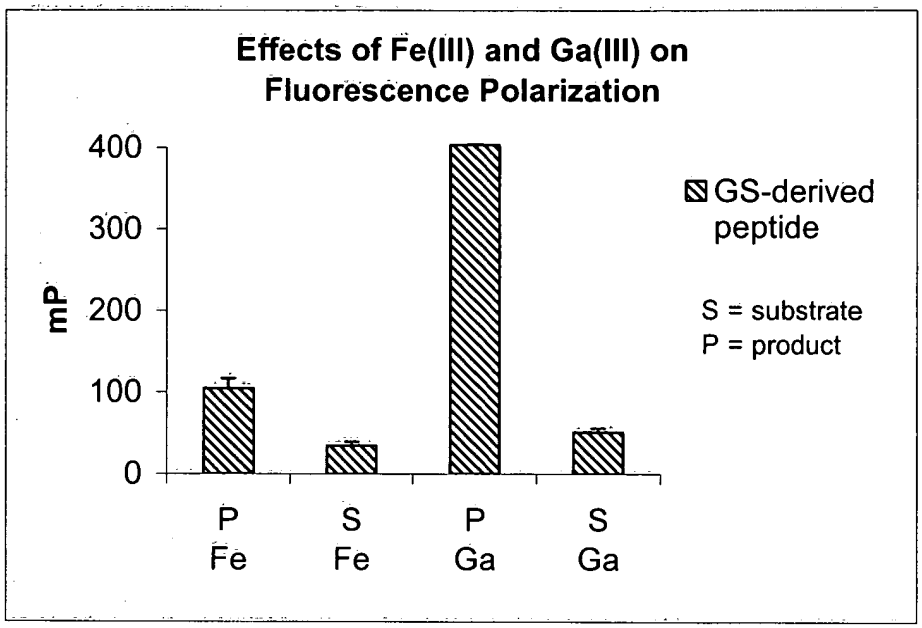

Figure 2

The "dynamic range" is determined by the difference between the degree of polarization produced by luminophore-associated product and substrate. In the configurations tested, the iron reagent provides a dynamic range of about 70 mP, and the gallium reagent provides a dynamic range of about 350 mP.[9] Thus, because it is much easier to measure a change of 350 mP than a change of 70 mP (since the larger change will be more significant relative to noise than the smaller change), it can take significantly less time to collect enough light to get good enough statistics reliably to measure the large change in polarization in gallium-based assays than the small change in polarization in iron-based assays.

Second, gallium-based polarization assays may be significantly more capable of detecting binding in assays involving mixtures of substrate and product (e.g., assays in which there is only intermediate enzyme activity) than iron-based polarization assays.

[9] The minimum possible dynamic range is 0 mP, and the maximum possible dynamic range is 500 mP, for randomly oriented molecules in a polarization assay. See, e.g. Application, page 52, lines 8-16. Thus, the dynamic range for Fe(III)-based polarization assays lies near the bottom of the possible range, and the dynamic range for Ga(III)-based polarization assays lies near the top of the possible range.

Such "intermediate enzyme activity" can be a common experimental situation, reflecting intermediate activity of the enzyme itself, intermediate activity of putative modulators of the enzyme (e.g., in a screening assay), and/or intermediate progress in reactions mediated by the enzyme (e.g., brought about by prematurely stopping the assay). The net polarization of a mixture of product and substrate depends on the separate polarizations of their associated luminophores, weighted in part by their relative intensities. Therefore, quenched luminophores, produced with the iron reagent, will contribute to the net polarization only minimally, if at all, because their luminescence emissions are quenched. Thus, the net polarization of a mixture in which bound luminophore is quenched by iron will arise mostly or exclusively from unbound luminophore, making corresponding binding assays insensitive to the binding that they are supposed to detect. In contrast, the net polarization of a mixture in which the luminescence of bound luminophore is enhanced by gallium will arise preferentially from bound luminophore, allowing more sensitive (rather than less sensitive) detection of binding in a mixture of substrate and product.

None of the references of record, including Nikiforov and Posewitz, teach or suggest these additional unexpected and patentable benefits of using a binding partner including gallium in polarization assays.

D. Summary of Experimental Results

The comparison of the iron reagent and the gallium reagent, presented above, indicates that the gallium reagent is dramatically superior to the iron reagent in at least three aspects. First, the gallium reagent, relative to the iron reagent, permits detectable emission of about one-hundred fold more light from a bound, exemplary luminophore.

Second, the gallium reagent has a large, near maximal dynamic range of polarization, while the iron reagent has a small, near minimal dynamic range of polarization. Third, the gallium reagent can better distinguish the existence of product in a mixture of substrate and product, relative to the iron reagent, since product bound to the gallium reagent will contribute preferentially more rather than preferentially less to the total polarization. Therefore, with the configurations tested, the use of the gallium reagent in place of the iron reagent converts a binding assay that effectively is inoperable into one that is very robust. None of the references of record, including Nikiforov and Posewitz, teach or suggest any of these unexpected and patentable benefits of using a binding partner including gallium in polarization assays.

The shortcomings of iron presented here are consistent with applicants' observation that there do not appear to be any non-gallium metal-based polarization assays. The Nikiforov patent does not disclose data in which a metal was used in a binding partner, nor to applicants' knowledge has Nikiforov published any papers showing such an assay. Furthermore, Caliper Life Sciences, the owner of the Nikiforov patent, does not appear to have introduced a product based on polarization assays using iron, or any other metal, judging from applicants' March 15, 2004, review of their website. Instead, Caliper has introduced an inhomogeneous assay, termed KiSS (kinase selectivity screening), which uses electrophoresis to separate phosphorylated and unphosphorylated substrate, prior to detection.[10] In contrast, applicants have developed a variety of commercial products that are now for sale for detecting the activity of over twenty different enzymes using gallium-based polarization assays.

[10] See KiSS™ Technology: A Simple, Affordable Approach to Kinase Selectivity Screening (visited March 15, 2004) <http://www.calipertech.com/products/kiss.shtml>.

In summary, the unexpected benefits and superior properties demonstrated by the claimed assay rebut any assertion of prima facie obviousness by the Examiner. Thus, for at least these reasons, applicants respectfully request withdrawal of the rejections under 35 U.S.C. § 103(a) and prompt allowance of the pending claims.

IV. Conclusion

Applicants believe that they have addressed all of the issues raised by the Examiner in the Office action, and that the application currently is in condition for allowance. However, if the Examiner has any questions or comments, or if a telephone interview would advance the prosecution of the application, the Examiner is encouraged to call applicants' undersigned attorney at the telephone number listed below.

Respectfully submitted,

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CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail, postage prepaid, in an envelope addressed to: Mail Stop RCE, Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450 on April 5, 2004.

Renee Knight